

CLAIMS

1. A method for inducing differentiation of pluripotent cells comprising the following steps (a) and (b):

5 (a) culturing the pluripotent cells in a medium comprising any one of the following growth factors (i) to (iii):

(i) acidic fibroblast growth factor, fibroblast growth factor 4, and hepatocyte growth factor;

10 (ii) acidic fibroblast growth factor, and growth factor(s) selected from activin A, epidermal growth factor, and  $\beta$ -nerve growth factor; and

(iii) fibroblast growth factor 4, and growth factor(s) selected from activin A and hepatocyte growth factor; and,

15 (b) culturing the cell cultured in step (a) in a medium comprising oncostatin M.

2. The method according to claim 1, wherein a gelatin-coated culture dish is used in step (a), and a collagen type I-coated culture dish or laminin-coated culture dish is used in step (b).

20 3. The method according to claim 1, wherein a collagen type I-coated culture dish is used.

4. A method for inducing differentiation of pluripotent cells comprising the following steps (a) and (b):

25 (a) culturing the pluripotent cells in a medium comprising at least one growth factor selected from retinoic acid, leukemia inhibitory factor, and hepatocyte growth factor; and,

(b) culturing the cell cultured in step (a) in a medium comprising any one of the following growth factors (i) to (iii):

30 (i) acidic fibroblast growth factor, fibroblast growth factor 4, and hepatocyte growth factor;

(ii) acidic fibroblast growth factor, and growth factor(s) selected from activin A, epidermal growth factor and  $\beta$ -nerve growth factor; and

35 (iii) fibroblast growth factor 4, and growth factor(s) selected from activin A and hepatocyte growth factor.

5. The method according to claim 3, wherein gelatin-coated

culture dishes are used in steps (a) and (b).

6. A method for inducing differentiation of pluripotent cells comprising the following steps (a) to (c):

(a) culturing the pluripotent cells in a medium comprising at least one of the growth factors selected from retinoic acid, leukemia inhibitory factor and hepatocyte growth factor;

(b) culturing the cell cultured in step (a) in a medium comprising any one of the following growth factors (i) to (iii):

(i) acidic fibroblast growth factor, fibroblast growth factor 4 and hepatocyte growth factor;

(ii) acidic fibroblast growth factor, and growth factor(s) selected from activin A, epidermal growth factor and  $\beta$ -nerve growth factor; and

(iii) fibroblast growth factor 4, and growth factor(s) selected from activin A and hepatocyte growth factor; and,

(c) culturing the cells cultured in step (b) in a medium comprising oncostatin M.

7. The method according to claim 5, wherein gelatin-coated culture dishes are used in steps (a) and (b), and a collagen type I-coated culture dish or laminin-coated culture dish is used in step (c).

8. A method according to any one of claims 1 to 7, wherein the pluripotent cells are derived from a mammal.

9. The method according to claim 8, wherein the mammal is a human, monkey, mouse, rat or pig.

10. A method according to any one of claims 1 to 9, wherein the pluripotent cells are embryonic stem cells, adult stem cells, mesenchymal stem cells, or umbilical cord blood cells.

11. A method for producing hepatocytes, wherein the method comprises steps (a) and (b) according to any one of claims 1 to 5, or steps (a) to (c) according to claim 6 or 7.

12. The method according to claim 11, wherein the hepatocytes are mature hepatocytes.

13. The method according to claim 11 or 12, wherein the pluripotent cells are derived from a mammal.

14. The method according to claim 13, wherein the mammal is a human, monkey, mouse, rat or pig.

15. A method according to any one of claims 11 to 14, wherein the pluripotent cells are embryonic stem cells, adult stem cells, mesenchymal stem cells, or umbilical cord blood cells.

16. A hepatocyte produced by a method according to any one of claims 11 to 15.

17. A therapeutic agent for a liver disease comprising the hepatocyte according to claim 16.

18. The therapeutic agent according to claim 17, wherein the liver disease is cirrhosis, fulminant hepatitis, biliary atresia, liver cancer, or hepatitis.

19. A kit comprising any one of the following (a) to (c):

(a) acidic fibroblast growth factor, fibroblast growth factor 4, and hepatocyte growth factor;

(b) acidic fibroblast growth factor, and growth factor(s) selected from activin A, epidermal growth factor, and  $\beta$ -nerve growth factor; and

(c) fibroblast growth factor 4, and growth factor(s) selected from activin A and hepatocyte growth factor.

20. The kit according to claim 19 further comprising oncostatin M.

21. The kit according to claim 20 further comprising at least one growth factor selected from the group consisting of retinoic acid, leukemia inhibitory factor, and hepatocyte growth factor.